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Short Communication

Prevalence of *Cryptosporidium spp*. in children with diarrhoea in the West Bank, Palestine

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Abstract
This study was conducted to investigate the prevalence of *Cryptosporidium spp*. in children (n=760) with diarrhea aged 1 month to 13 years, living in urban areas (n=234), rural areas (n=394) and refugee camps (n=132). Samples were collected, stained by modified acid fast stain, and examined microscopically for oocysts. The overall prevalence was 11.6% (88/760). The prevalence was higher in refugee camps at 12.9% (17/132) and in rural areas at 12.2% (48/394) as compared to 9.8% (23/234) in urban areas. According to age, the prevalence in age group I (<5 years) was significantly high (P<0.05) at 14.4% (67/464) as compared to 7.7% (15/195) in age group II (5-10 years) and 5.9% (6/101) in age group III (10-15 years). Our findings indicate that the prevalence of *Cryptosporidium spp.* is high when compared to that in developed countries.
Key Words: Cryptosporidium spp., Oocysts, Modified acid fast stain, Diarrhea.

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Introduction

Cryptosporidium is an intracellular protozoan parasite that has emerged as a major cause of diarrhea in humans and animals [1]. It is a coccidian parasite belonging to the Phylum Apicomplexa [2]. Diarrhoeal diseases are extremely common in developing and developed countries. They are responsible for morbidity and mortality of millions of individuals every year [3]. Cryptosporidiosis is endemic in developing countries due to poor sanitation, crowding, and malnourishment [4]. *Cryptosporidium* is considered one of the most important enteric pathogens with worldwide distribution [5]. *Cryptosporidium* causes severe diarrhea in patients with immunodeficiency, and may be life threatening in patients with AIDS. In AIDS patients, the incidence and severity of cryptosporidiosis increases as the CD4 lymphocyte cell count falls below 200 cells/ul [6].

The rate of infection is predicted to be higher in malnourished children of developing countries [7,8,9]. Epidemiological data on the prevalence of *Cryptosporidium* infections seem to be very sparse in most developing countries including Palestine. The prevalence of *cryptosporidium* in children with diarrhoea in the neighboring countries was found to be 8.8% in Iraq [10], 1.5% in Irbid, a city in Jordan, [11] and 16.6%, 11.6%, 27.9% in Egypt for the years 1986, 1987 and 1996 respectively [12]. In Rawalpindi, Pakistan, the prevalence was found to be 10.3% [13] and 7.3% in India [14]. A study conducted at Caritas Baby Hospital in the city of Bethlehem, Palestine, revealed a prevalence of 13.5% [7]. In Gaza-Palestine, the prevalence was found to be 14.6% with a high mortality rate of 38% [15].

Materials and Methods

Study design, site and population

This is a prospective study about the epidemiology of *Cryptosporidium spp*. in the West Bank, Palestine. The prevalence of *Cryptosporidium spp*. was determined in children with diarrhoea (n=760) ages one month to 15 years of age, admitted to major community hospitals in the West Bank, Palestine. The samples were collected between September 2003 and November 2004. The distribution of specimens to the different areas was as follows: urban areas (n= 234), rural areas (n=394), refugee camps (n=132) and controls (n=62).

Sample Collection and Processing

Fecal samples were collected in a dry, clean, leak-proof plastic container. Each sample was labeled with the child's name, gender, and age. Additional information and demographics related to each sample were obtained from the hospital. Informed consent was obtained from the parent or guardian of all children before enrollment in the study. Matched controls were obtained from children without diarrhoea and treated exactly in the same manner as the other specimens. Sample collection, storage, and transport were carried out according to the specifications of the Center of Disease Control [16,17]. The stool samples were concentrated using the ethyl acetate sedimentation method as recommended by the Center of Disease Control [16] and stained by the modified acid fast stain procedure outlined by Garcia [18].

Statistical Evaluation

Chi square (χ^2) was used to detect significant differences between the various groups at 5% level of significance. SPSS 11.0 for Windows was used to do the statistical analysis.

Results

Fecal samples were taken from 760 children with diarrhoea, one month to 15 years old. A total of 234 children (30.8%) were from urban areas, while 394 children (51.8%) were from rural villages and 132 children (17.4%) were from refugee camps (Table 1).

Cryptosporidium spp. was detected by the modified acid fast stain as shown in Figure 1, in a total of 88 children (11.6%). Of these, 67 children (14.4%) were < 5 years old, 15 children (7.7%) were 5 to 10 years old and 6 children (5.9%) were 11 to 15 years old (Figure 1). *Cryptosporidium* oocysts were seen in 2 children (3.2%) of the normal control group.

The prevalence of *Cryptosporidium spp*. in the children living in urban centers, rural villages and refugee camps was as follows: 23 of 234 (9.8%), 48 of 394 (12.2%) and 17 of 132 (12.9%) respectively (Table 1).

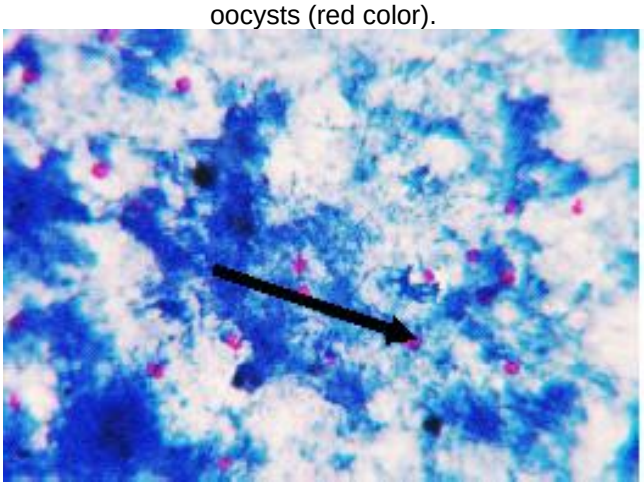
Discussion

Cryptosporidiosis is endemic in developing countries due to poor sanitation, crowding, and malnourishment [4]. *Cryptosporidium* is considered one of the most prevalent enteric pathogens with world-wide distribution [5].

Table 1. Represents the total number of specimens collected and distributed according to area of residence and age groups. The number of positive samples and their percentage according to each category is also shown.

Category	Total number	*Cryptosporidium* spp. Positive	% positive
AGE			
< 5 years	464	67	14.4
5-10 years	195	15	7.7
11 – 15 years	101	6	5.9
Area of residence			
Urban centers	234	23	9.8
Rural villages	394	48	12.2
Refugee camps	132	17	12.9
Control group	62	2	3.2

Figure 1. Modified acid fast stain on a specimen concentrated with the ethyl acetate procedure, showing acid fast positive *Cryptosporidium spp.* oocysts (red color).



The purpose of this study was to determine the prevalence rate of *Cryptosporidium spp.* among Palestinian children with diarrhoea living in different areas of the West Bank.

The prevalence rate of *Cryptosporidium* in children with diarrhoea in the West Bank was relatively high (11.6%) compared to 3.2% in the control group. Previous prevalence studies for this parasite revealed a prevalence rate of 13.5% in children with diarrhoea admitted to Caritas Baby Hospital, Bethlehem, West Bank [7]. In a similar study conducted in the Gaza Strip (the southern

part of Palestinian territory), the prevalence rate of *Cryptosporidium* was (14.6%). The results were consistent with those obtained in Egypt in 1987 with a prevalence rate of (11.6%). In Rawalpindi, Pakistan, prevalence rates of 10.3% in diarrhoeic children and 3.3% in the control group were reported [13]. In Iraq, the prevalence rate was 8.8%, much lower than that obtained in this study for children under five years old with gastroenteritis [11]. In Irbid, Jordan, the prevalence rate for *Cryptosporidium* was 1.5% among children under five years of age [10]. This difference could be attributed to higher standards of living, better hygiene, better socioeconomic class, and cleaner water.

The high prevalence rate of *Cryptosporidium* in the West Bank may be attributed to wastewater disposal. Many of the rural areas and practically all refugee camps do not have proper sewage disposal. They rely on small porous cesspits that fill quickly and very often overflow to the streets. A recent survey by the Palestine Central Bureau of Statistics [19] indicated that 71.2% of people living in the southern part of the West Bank, 61.7% in the northern part, and 56.1% in the central part do not have sewage disposal systems. Moreover, domestic rain wells are deeper than cesspit levels in nearly 80% of these regions [19]. Ultimately, drinking water supplies become contaminated and form a health hazard, increasing the incidence rates and facilitating the transmission of this pathogen. Reports by the Palestinian Ministry of Health [19] revealed that water contamination rates range from 13.4% to 35.8% in the different districts of the west Bank. Similar findings regarding water contamination rates were found in 2005 [21]. The higher prevalence rate of cryptosporidiosis in refugee camps is due to the poor sanitary conditions in the camps, contaminated drinking water, and lack of sewage systems [20]. The highest prevalence rate of cryptosporidiosis was found in children younger than 5 years age (14.4%) as compared to that in children 5 to 10 years old (7.7%) and in children 11 to 15 years of age (5.9%). This significant difference ($P<0.05$) could be attributed to the poor living conditions of these children, as well as a lack of self-awareness, personal hygiene and cleanliness at this critical age. These conditions place the children in a high risk group to contract cryptosporidiosis at higher rates [9,20,21].

Our findings suggest the necessity to implement newer regulations to implement the routine testing for *Cryptosporidium* on all children with diarrhoea. In addition, measures should be taken to ensure the delivery of clean, uncontaminated drinking water to people living in refugee camps and rural areas, as well as to improve their living conditions and develop adequate sewage systems in these areas.

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Conflict of interest: No conflict of interest is declared.